

Amendments to the Specification

Paragraph at page 2, line 34 to page 3, line 3:

It is preferred that the isolated polynucleotides of the claimed invention consist[[s]] of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16, that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, and 17. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequences of at least ~~one of~~ 60 (preferably at least ~~one of~~ 40, most preferably at least ~~one of~~ 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16, and the complement of such nucleotide sequences.

Paragraph at page 3, line 34 to page 4, line 5:

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a delta-6 desaturase or sphingolipid desaturase polypeptide gene, preferably a plant delta-6 desaturase or sphingolipid desaturase polypeptide gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least ~~one of~~ 40 (preferably at least ~~one of~~ 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a delta-6 desaturase or sphingolipid desaturase amino acid sequence.

Paragraph at page 4, line 34 to page 5, line 5:

Figures 1A-C show ~~Figure 1 shows~~ a comparison of the amino acid sequences of the Florida bitterbush delta-6 desaturase from clone pps.pk0011.d5: fis (SEQ ID NO:2, denoted [SIN 2]), the soybean sphingolipid desaturase from clone ssl.pk0017.b4: fis (SEQ ID NO:8, denoted [SIN 8]), the borage delta-6 desaturase having NCBI General Identification No. 2062403 (SEQ ID NO:11), corn sphingolipid desaturase from clone cde1c.pk001.o8: fis (SEQ ID NO:4, denoted [SIN 4]), wheat sphingolipid desaturase from clone wre1.pk0004.c7: fis (SEQ ID NO:10, denoted [SIN 10]), wheat delta-6 desaturase-like protein having NCBI General Identifier No. 4104056 (SEQ ID NO:12), soybean sphingolipid desaturase from the contig assembled of clones sfl1.pk0012.c5 and sfl1.pk0031.d11 (SEQ ID NO:6, denoted [SIN 6]), and sunflower having NCBI General Identifier No. 1040729 (SEQ ID NO:13).

Paragraph at page 5, line 23 to page 6, line 6:

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least ~~one of~~ 60 contiguous nucleotides, preferably at least ~~one of~~ 40 contiguous nucleotides, most preferably ~~one of~~ at least 30 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 16, or the complement of such sequences.

Paragraph at page 6, lines 27-37:

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least ~~one of~~ 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

Paragraph at page 7, lines 1-30:

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for

glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least ~~one of~~ 60 (preferably at least ~~one of~~ 40, most preferably at least ~~one of~~ 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16, and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide such as a delta-6 fatty acid desaturase and/or a sphingolipid desaturase in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial, or viral) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

Paragraph at page 8, line 29 to page 9, line 13:

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) J. Mol. Biol. 215:403-410 ~~Altschul et al. (1993) J. Mol. Biol. 215:403-410~~; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or

bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

Paragraph at page 13, line 16 to page 14, line 7:

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide. The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide of a

gene (such as delta-6 desaturase or sphingolipid desaturase) preferably a substantial portion of a plant polypeptide of a gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least ~~one of~~ 60 (preferably at least ~~one of~~ 40, most preferably at least ~~one of~~ 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, and 16, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a polypeptide.

Paragraph at page 19, lines 10-26:

cDNA clones encoding delta-6 desaturase or sphingolipid desaturase were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410 ~~Altschul et al. (1993) *J. Mol. Biol.* 215:403-410~~; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

Paragraph at page 20, line 29 to page 21, line 9:

Figures 1A-1C present ~~Figure 1 presents~~ an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, and 10 and the borage sequence (SEQ ID NO:11), wheat sequence (SEQ ID NO:12), and sunflower sequence (SEQ ID NO:13). The data in Table 5 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, and 10, and the borage sequence (NCBI General Identifier No. 2062403, SEQ ID NO:11), wheat sequence

(NCBI General Identifier No. 4104056, SEQ ID NO:12), and sunflower sequence (NCBI General Identifier No. 1040729, SEQ ID NO:13). The data in Table 5 represents a calculation of the percent identity of the amino acid sequence set forth in SEQ ID NOs:2, 4, 6, 8, and 10 and the delta 6 desaturase borage sequence (SEQ ID NO:11) and the wheat and sunflower sphingolipid desaturases (SEQ ID NOs:12 and 13, respectively).